Archaeology of the invisible: the scent of Kha and Merit

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Abstract

The burial assemblage of Kha and Merit discovered in 1906 and now preserved at the Museo Egizio in Turin constitutes the most abundant, complete, and well-preserved non-royal Egyptian burial assemblage ever found. The discoverer of the tomb and director of Museo Egizio, Ernesto Schiaparelli, limited investigations to a few non-unique items, leaving most of the assemblage untouched. His decision has enabled contemporary scholars to study such exceptional archaeological finding with non-invasive approaches that could not be foreseen at the time. Here we show that the volatile compounds emitted by organic materials contained in vessels can be analysed and detected directly in museums by a transportable mass spectrometer (selected-ion flow-tube mass spectrometry, SIFT-MS). The interpretation of the mass spectrometric data, supported by statistical data analysis, shed new light on the chemical nature of the organic materials preserved in 50 vessels of the Museo Egizio in a completely non-destructive way.

Our study proves the reliability of SIFT-MS as a fast-screening method for archaeometric investigations directly in museum, providing the unique possibility to complement, confirm and extend the archaeological research on the use and purpose of archaeological vessels and jars.

1. Introduction

On 15 February 1906, the Missione Archeologica Italiana (Italian Archaeological Mission) led by Ernesto Schiaparelli, the director of the Museo Egizio in Turin, discovered the underground tomb of two high-ranking individuals in the Deir el-Medina Necropolis (Luxor, West Bank): the "chief-of-works" (i.e., *foreman*) Kha and his wife, Merit, who lived during the second half of 18th dynasty (about 1450-1400 BCE) [1].

The tomb (labelled TT8) had never been plundered since it had been sealed by a landslide in ancient times. Schiaparelli found the funerary chamber full of over 440 objects - painted wooden boxes, furniture, pottery, metal bowls, tools, textiles, alabaster jars and food. Today this finding still represents the most abundant and complete non-royal burial assemblage ever found in Egypt [2].

In 2017, the Museo Egizio started the "TT8 Project", a research program of archaeometric investigations in collaboration with an international community of heritage scientists, Egyptologists, restorers, archivists, and archaeologists. The project aims at publishing a digital data repository and a full study of TT8, exploiting archaeological, historical, cultural, and conservation data, for the bicentennial of the collection in 2024 (the Drovetti collection arrived in Turin in 1824, forming the first basis of the Egyptian Museum). The research potential is made even more significant by the pristine state of the objects. In fact, Schiaparelli showed a farsighted approach to conservation in limiting the invasive investigations to a small group of a few non-unique items, while leaving the rest of the assemblage untouched. This attitude was unusual for the scientific community of his time.

In the framework of the "TT8 Project", twelve research groups are currently studying the various classes of objects and characterizing their constituent materials or their contents. The museum policy for non-invasive approaches entails the use of cutting-edge instruments and methodologies of analysis.

In this paper, we present the first extended on-field application of a completely non-destructive protocol based on selected-ion flow-tube mass spectrometry (SIFT-MS) to characterize organic materials in archaeological objects. The protocol was previously optimized and validated at laboratory scale, first on reference materials and then on small samples of amorphous archaeological residues [3, 4].

Until now, the most reliable information on the molecular composition of archaeological organic materials has been provided by analyzing micro-samples using protocols based on chromatography and mass spectrometry (GC-MS and HPLC-MS analysis) [5-8]. Although these micro-invasive and destructive approaches are highly effective, they are difficult to implement when dealing with a wide and heterogeneous collection, such as the one in the Museo Egizio, where characterizing the organic materials in a high number of objects is crucial in order to obtain representative information, and to define the best storage, maintenance, and display strategies. Moreover, GC-MS and HPLC-MS are not applicable when it is not possible or not opportune to collect a sample, as it happens for sealed containers or unique and precious archaeological findings

In previous investigations of the Kha and Merit funerary assemblage, the painted layers of the grave goods were investigated by non-invasive multispectral imaging, which included in-situ spectroscopic techniques as Raman, mid-infrared, near-infrared, and XRF spectroscopies [9, 10]. Unfortunately, non-invasive vibrational spectroscopies were not sufficient to identify and to chemically resolve the complex mixtures of organic materials contained in the majority of the vessels constituting the funerary set (data publication in progress). In another study, significant amounts of organic materials in seven sealed vessels were revealed by high-resolution neutron radiography and tomography [11, 12]

In the experiments here described, we investigated about 50 objects from the burial assemblage of Kha and Merit in situ in the museum premises, using a transportable SIFT-MS system. This instrumentation enabled us to perform non-invasive real-time analyses of volatile organic compounds (VOCs) at trace levels directly on the headspace of the archaeological objects.

In the last years VOCs analysis has been applied in the field of cultural heritage in few selected cases. In particular, in archaeological contexts, solid phase micro extraction-gas chromatography mass spectrometry (SPME-GC-MS) has only seldom been applied, for examples for the analysis of beeswax and natural resins [13-21], compared to its wide application for the analysis of VOCs emitted from plastic materials, such as nitrocellulose and polyurethane, and for the study of the degradation of paper. Even though the analysis of VOCs could be applied in museum environments by offline sampling, representing a good alternative to the destructive sampling of an object, the

following analysis must be performed off-line on laboratory instruments. Thus, SPME-based techniques are difficult to apply to a high number of samples and objects in extensive diagnostic campaigns. Moreover, the molecular profile of VOCs determined by SPME-GC-MS can differ from that of the sampled headspace, because biases are introduced according to the stationary phase used in SPME, the duration and the temperature of the sampling step, and the concentration of the different type of VOCs in the samples. Finally, the reproducibility of sampling with SPME devices is relatively low. For all these reasons, SPME-GC-MS approach is affected by severe limitation when the target is to study heterogenous objects, with unpredictable concentrations of VOCs, and completely unknown composition.

SIFT-MS is a fast direct mass spectrometric technique that allows us to perform real-time mass spectrometric analyses of VOCs by controlled ultra-soft chemical ionization. In detail, the sample headspace is directly introduced in the mass spectrometer, preventing any discrimination of the analytes; the VOCs are then ionized using different reagent ions (H_3O^+, O_2^+, NO^+) that yield distinct mass spectra, providing complementary information on the chemical species and overcoming the limitations due to the absence of a chromatographic separation. The identification of the chemical species in the SIFT-MS mass spectra is performed by the identification and evaluation of the ratios between specific m/z values obtained with multiple reagent ions; the combination of H_3O^+, O_2^+, NO^+ is generally sufficient to discriminate a large number of chemical species. Due to the standard conditions used in the ultra-soft ionization process and the different behaviors of the analytes during the ionization, these values are extremely reproducible and specific and can be used to accurately identify different classes of compounds. Moreover, since the chemical structure of the analytes plays a fundamental role in the formation of specific ionization products, the SIFT-MS performed using multiple reagent ions could also allow to differentiate between isobaric isomers. Finally, SIFT-MS can be also applied for direct quantitative analysis [22].

The analytical protocol presented in this work allowed us to carry out the first experiments in-museum on a large scale of archeological artefacts thanks to the previous collection of a data base of SIFT-MS mass spectra of organic materials that can be encountered in archaeological objects [3, 4]. The use of SIFT-MS in combination with multivariate analysis permitted us to identify different similarities

in the VOCs composition of the archaeological objects, allowing us to hypothesize the origin of most of the amorphous materials constituting or contained in the objects. Finally, to validate the analytical approach used in-museum for the first time, the hypotheses formulated by interpreting the SIFT-MS of eight objects were validated by comparison with those obtained from the characterization of microsamples collected from same artifacts using a consolidated and fully validated GC/MS approach based on saponification, extraction, and derivatization, and analytical pyrolysis coupled with GC-MS (Py-GC-MS) [5, 23]. The comparison of the markers used for the identification of the different materials with these analytical approaches is reported in the Supporting Information. Notably, the comparison of results obtained with micro-destructive techniques is fundamental for future applications of SIFT-MS. On the other hand, it is very important to choose a group of objects where the sampling does not cause any alteration or damage to the integrity of artifacts, in terms of nature, abundance, morphology and accessibility of the organic residues, and any interference in future studies.

The success of this survey proves the reliability of SIFT-MS as a fast-screening method for archaeometric investigations directly in museum, providing a further advancement with respect to our previous pilot studies [3, 4, 24]. By an archaeological and historical point of view, the interpretation of the mass spectrometric data, supported by statistical data analysis, and confirmed by GC/MS analysis, shed new light on the chemical nature of the preserved material in the Museo Egizio in a completely non-destructive way.

2. Materials and methods

2.1 Archaeological objects, samples, pre-concentration of the VOCs, and in-situ analysis

For this study, 46 objects from tomb TT8 were selected. The complete list of the objects, together with the inventory numbers is reported in Table 1. The objects were intact and even if there is no information in the Museum track record and, to date, no evidence has been found on the artworks, we cannot exclude that ordinary conservation treatments have been carried out in the first half of the twentieth century. Starting from 1980, two treatments have been conducted on S.8516 and S.8526 (re-adhesion of fragments with synthetic polymers) and the consolidation of the textile top part of amphora S.8619 (linin in nylon net with couching stitch). Notwithstanding this, the presence of none or few conservation treatments allows us to associate the VOCs emitted from the objects' profiles to their original content or composition.

One week prior to each analysis campaign, the objects were isolated from the environment using customized inert bags directly inside the showcases, and thus with minimal inconvenience for the visitors to the exhibition (**Figure 1**).



Figure 1 – Goods from the burial assemblage of Kha and Merit preserved at the Museo Egizio in Turin prepared for the SIFT-MS analysis.

The objects were inserted into two different types of inert bags, one type cut from Nalophan bag rolls (one with 15 cm caliber and one with 30 cm caliber), and another consisting in inert zip-lock bags (45 cm x 35 cm flat). Both types of bags were hermetically sealed according to the procedure reported in [25].

A customized auto-sealed silicon septum was used to perform the analyses by directly inserting the SIFT probe needle inside each bag, thus allowing the analysis of the material's headspace (volatiles in the gaseous phase in equilibrium with the matrix to be sampled) and preventing any dilution of the analytes and possible environmental contamination. Moreover, the scans performed on the VOCs emitted by the objects and pre-concentrated in the bags were very fast (around one minute). **Figure 2** shows examples of the type of measurement approaches performed on a selection of objects.



Figure 2 - Different approaches used for the mass spectra acquisition of the headspace of the materials inside the objects.

Most of the object was sealed and due to the museum policy it was not possible to open them; only for few objects the lids were removed and left in the bag to increase the volatile organic compounds

released in the sealed bag (S.8483, S.8484). Objects S.8224, S.8323, S.8526 did not fit in the bags and for this reason we decided to isolate only the top of the object. Finally objects S.8356, S.8467, S.8516 were directly analyzed by inserting the SIFT probe inside the necks of the vases, as close as possible to the inner content.

The analyses were performed in two different analysis campaigns (July and November 2019). The analyses of objects S.8619 and S.0620 were performed in triplicates (the spectra were acquired one after the other for three consecutive acquisition runs) to prove the intraday reproducibility. As for the interday reproducibility, the replicates did not show significative differences. The same objects (S.8619 and S.8620) were re-sealed and analyzed in both the campaigns to evaluate the interday reproducibility, which proved very good in terms of semi-quantitative and qualitative analysis.

A set of eight samples was used to perform micro destructive analysis. In detail, some samples were directly sampled from the objects, namely from S.8239 (collected with a scalpel from the lump), S.8526 (fragment fallen out during analyses), and S.8356 (collected from the abundant residue inside the amphora). Conversely, samples from S.8441, S.8442, S.8443, S.8445, and S.8447 were obtained as dabbed material from the corresponding objects using a nalophan sheet, and recovered using a mix of acetone, methanol, and dichloromethane. The latter were analyzed only by GC-MS, while samples S.8239, S.8526, and S.8356 were analyzed both by Py-GC-MS and GC-MS.

2.2 Blanks

For each type of bag and for each exposure case, an analytical environmental/procedural blank was registered by sealing an empty bag (c.a. 2 L) and placing it next to the archaeological objects for the whole sampling time (one week). For those vases analyzed by inserting the SIFT probe inside their necks, the air of the analysis rooms was employed as environmental blank.

2.3 Gas Chromatography/Mass Spectrometry (GC-MS)

All the samples (c.a. 200 µg of solid, or the whole recovered extract after drying) were subjected to microwave assisted saponification with a 600 µL of solution of 10% KOH in EtOH at 80°C and 200W for 60 min [26]. After the saponification, the samples were purified using a multi-step extraction with

n-hexane for the neutral compounds and diethyl ether after acidification with hydrochloric acid (6 M) for the acidic species. The two extracts were admixed together and were dried under N_2 stream and derivatized with 20 µL of BSTFA containing 1% trimethylchlorosilane, 150 µL of isooctane and 5 µL of tridecanoic acid solution at 60°C for 30 min. 5 µL of hexadecane solution were added before the injection. GC/MS analyses were performed using an Agilent Technologies 6890N gas chromatograph with a split/splitless injection port and coupled with a 5975 mass selective single quadrupole mass spectrometer (Agilent Technologies, USA). The full GC/MS conditions are reported in [27]. The analytes were identified by comparison with mass spectra libraries (NIST 20), analytical standards and reference materials.

2.4 Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC-MS)

The analyses were performed using a multi-shot pyrolyzer EGA/PY-3030D (Frontier Lab, Japan) coupled with an 8890 gas chromatograph, combined with a 5977B mass selective single quadrupole mass spectrometer detector (Agilent Technologies, US).

The samples S.8239, S.8526, and S.8356 (20-50 µg) were placed in a stainless-steel cup with 5 µL of hexamethyldisilazane (HMDS) before the pyrolysis. The pyrolysis was performed at 550 °C, with the pyrolyzer interface set at 280 °C. The full GC/MS conditions are reported in [28, 29].

2.5 SIFT-MS acquisitions

The SIFT mass spectrometric acquisitions were performed using a Voice 200ultra instrument (SYFT Technologies, New Zealand). The full scans were executed using H_3O^+, NO^+, and O_2^+ as reagent ions, and nitrogen as the carrier gas (flow 25 mL/min, purity 99.999%) [24, 30, 31]. The ion products of the chemical ionization reactions were scanned by the second quadrupole in the range m/z 15-400. The acquisition time for each mass was set at 100 msec with steps of 1.0 m/z. Data acquisition was performed using the LabSyft v. 1.6.2 (SYFT Technologies).

2.6 Data analysis

The data analysis was performed using LabSyft Pro 1.8.1 (SYFT Technologies). For each mass spectrum the corresponding blanks and the signals deriving from the excess reagent ions were subtracted.

The PCA was performed using the ion intensities of the three mass spectra acquired for each object as variables, after the subtraction of the blanks and the excess of the reagent ions from each mass spectra. The mass spectra were further subtracted from the ions specific to acetone, acetaldehyde, and acetic acid since their secondary chemistry in SIFT-MS acquisitions leads to the formation of specific ions with relatively high intensities, which are not diagnostic for the identification of the materials. Sample S.8523 was removed from the database since the mass spectra were characterized only by the presence of the ions characteristic of acetophenone.

For each sample, the mass spectra of each reagent ion were row-normalized by expressing the relative abundances for each species as a percentage of the total intensity obtained by summing the intensities of all the ions considered. The PCA on the covariance matrix of the SIFT-MS analysis data was performed with Xlstat 10.0 (Addinsoft).

Cluster analysis was performed by first labelling each sample according to a first, qualitative interpretation of the mass spectra, as reported in Table 1 (PCA Label column), and then by applying the cluster analysis algorithm using BioVinci 1.1.5 (BioTuring Inc.) according to the t-distribution (95% confidence level).

3. Results and Discussion

Due to the large number of mass spectra acquired during the campaign, the data were processed by principal component analysis (PCA) to identify and classify the samples based on the mass spectrometric features. PC1 and PC2 only accounted for 45% of the variance of the sample set. However, by labelling each object accordingly to a preliminary mass spectra interpretation, and then applying a cluster analysis algorithm based on the t-distribution, we were able to visualize and identify three different clusters.

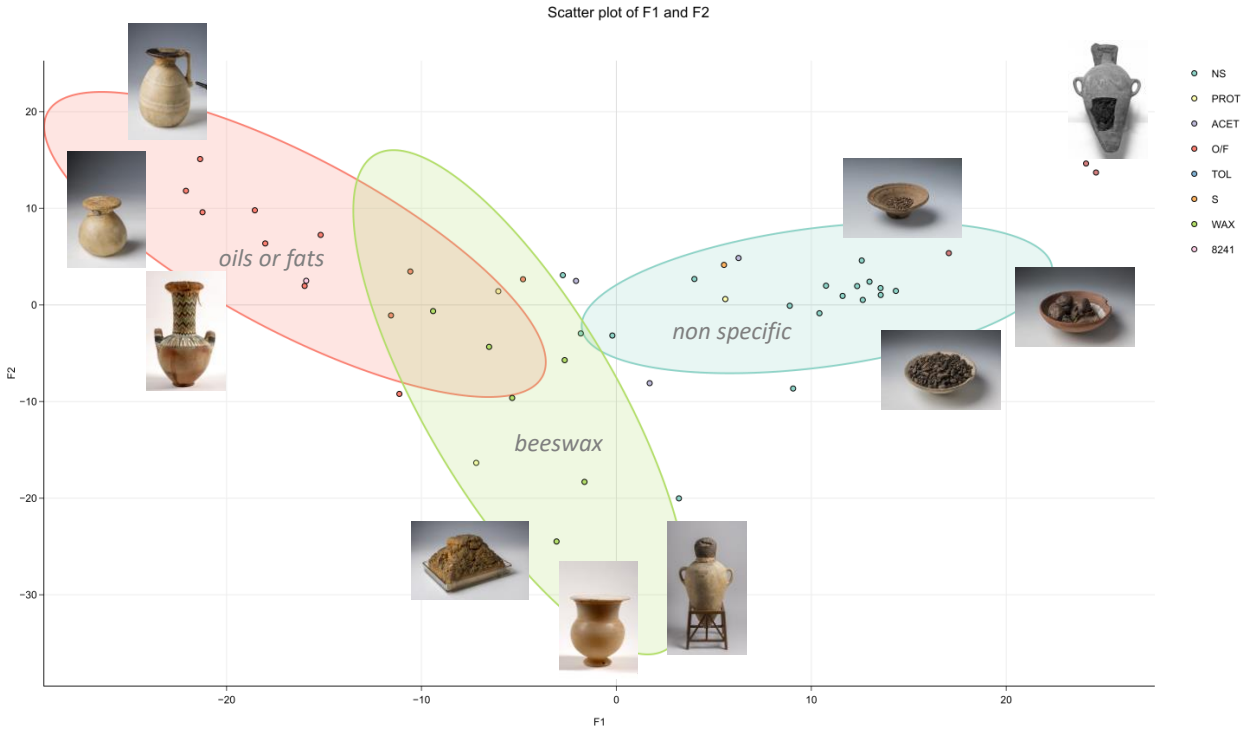


Figure 3 - Scatter plot of the first two PCs calculated by PCA analysis of the mass spectral data after suitable normalization. The labels applied to each object prior to cluster analysis and shown in the legend are: NS = profile not specific; PROT = protein material; ACET = acetone/acetic acid or acetaldehyde; TOL = toluene as main peak; O/F = Oil or fat material; WAX = beeswax). Pictures of selected objects are placed next to the corresponding clusters.

The interpretation of the SIFT-MS mass spectra was used as a basis to define the following clusters as: lipid material, probably oils or fats (O/F); beeswax (WAX); blank level or unspecific (NS).

Inside the NS group, several samples showed specific mass spectrometric features and were labeled accordingly: amines possible related to proteins (PROT) and markers of sulfur species (S).

We discuss the results in relation to the mass spectrometric similarities highlighted by the PCA analysis, taking into account any correspondence between the vessels' contents, their position in the tomb, and archaeological records. All the mass spectra significantly different from the blanks together with the most intense ions used for the mass spectra interpretation are reported in the Supporting Information file.

The two objects (S.8526 and S.8323) outside the three clusters located at positive values of both the PCs, as shown in **Figure 3**, were both characterized by the presence of high amounts of toluene. The SIFT-MS mass spectra obtained for amphora S.8526 are reported in **Figure 4**.

By looking at the mass spectra in detail, the SIFT-MS analysis performed on amphora S.8526 enabled us to identify the ions characteristic of several amines, including ammonia, methylamine, dimethylamine, diethylamine, trimethylamine, and dimethylnitrosoamine. These compounds may be related to products of the biodegradation of proteins [32]. Relatively high amounts of trimethylamine, together with dimethylnitrosoamine are specific for dried fish [33-35]. The concomitant detection of monoterpenes and of a relatively high abundance of toluene, together with benzene, ethylbenzene, and benzoic acid suggests the co-presence of a plant aromatic resin, probably from the genus *Styrax* [36]. Due to the fragile nature of the content of the amphora, visible through a dent produced during an analytical campaign performed by Schiaparelli himself, different erratic samples were available for micro-destructive analysis, whose results are presented in **Figure 4**. In particular, the Py-GC-MS analysis features pyrrole, toluene, and diketopiperazines, pyrolysis products that can be associated to the presence of proteins [37]. Owing to the high abundances of fatty acids, in order to obtain more detailed information on the lipid portion of the sample, we performed GC/MS analysis after saponification, extraction, and derivatization reaction. The GC/MS analysis allowed us to confirm the presence of an aromatic resin by the identification of benzoic acid, vanillyl alcohol, 4-hydroxybenzoic acid, and p-hydroxycinnamic acid as the most abundant chemical species [38]. The presence of pristanic and phytanic acids [39, 40], together with the presence of dicarboxylic acids in the range C_4-$C_{14,}$ deriving from the oxidation of omega-fatty acids, and fatty acids with an odd number of carbon atoms (C_{15} and C_{17}), characteristic of animal fats, allowed us to further confirm the hypothesis formulated based on SIFT-MS analysis on the possible presence of fish.

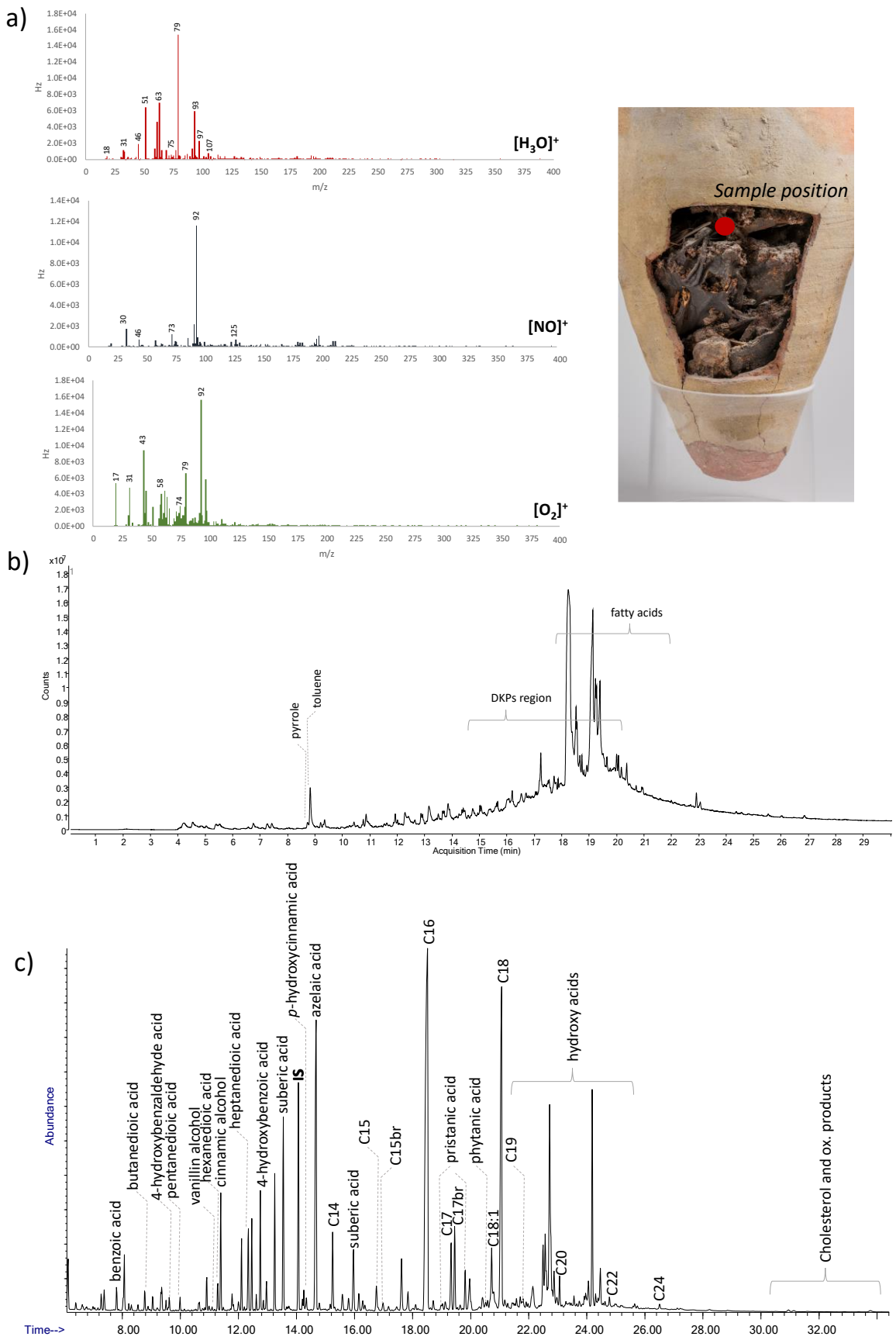


Figure 4 – SIFT-MS mass spectra (a), Py-GC-MS (b) and GC-MS (c) chromatograms obtained for a sample collected from the object S.8526

The cluster encompassing the objects with the nonspecific profile (NS) contains all the cups and bowls containing fruit/vegetables or food residues, which have similar yet unspecific SIFT mass spectra.

The SIFT-MS analyses performed on the headspace of some cups, one bowl and an amorphous material of unknown origin featured furan and furfural derivatives, ascribable to natural degradation products from saccharide or polysaccharide materials [41-43], hydrocarbons in the C_9-C_{15} range, and aliphatic aldehydes in the C_5-C_{10} range. Such aldehydes are common in several fruits and other plant parts [44, 45], and account for the fruity aroma inside the museum showcases. Unfortunately, these markers are not sufficiently specific to ascertain the goods that these vessels once contained. Seven samples contained one or two major VOCs in their spectra, such as acetic acid, acetone, toluene, acetophenone, and/or acetaldehyde, whose characteristic m/z values dominate the mass spectra and possibly hinder peaks ascribable to other molecular markers. For instance, the mass spectra of the headspace of sample S.8523 were dominated by the peak of acetophenone, an oxidation product of several fruits and vegetables, and whose origin is thus hard to establish [46].

Finally, among these samples, cups S.8251 and S.8321 and amphora S.8224 showed further peculiar mass spectrometric features, absent in any other mass spectra in the NS cluster. The VOC profiles of cups S.8251 and S.8321 both featured traces of ammonia, methylamine, and dimethylamine, pointing to the presence of proteins. In addition, the headspace of S.8321 contained traces of trimethylamine and dimethylnitrosoamine, markers of dried fish, similarly to S.8526. These results are consistent with the importance of fish in the diet of the ancient Egyptians, and its reference in the lists of provisions for the village of Deir el-Medina. Moreover, fish (particularly of the genus *Tilapia*) were considered a symbol of rebirth and thus an appropriate gift for the deceased [47].

The mass spectra obtained for the headspace of amphora S.8224 were also characterized by specific ions, not present in the other samples in the NS cluster. The data interpretation enabled us to identify furan, 2-methyl furan, 2-ethyl furan, 2-pentyl furan, furfural, 5-methyl furfural. These species are degradation products of saccharide or polysaccharide materials, such as the linen cloth plugged inside the neck [41-43]. The additional presence of 3-methylbutanal, pentanal, hexanal,

heptanal, pentanoic and hexanoic acid, hexanol, heptanol, octanol, linalool, and benzaldehyde suggests that these latter aldehydes and alcohols originated from the amorphous material in the jar, identified as flour [48-50]. All these species are common in the VOC profiles of oat, buckwheat, and barley. Various sulphur-containing compounds were detected, the most abundant being carbon disulphide, dimethyl sulphide, ethyl-methyl sulphide, diethyl sulphide, methyl propyl sulphide, and allyl-methyl-sulphide. These species are formed from the maturation/maceration of cereals with a high content of amino acids and metabolites bearing a sulphur heteroatom [51]. Considering the whole profile, the material is likely to be barley [51-53].

In ancient Egypt, flour was not just a commodity, but also a medium of exchange, due to the absence of a monetary system to pay salaries [54]. Barley flour was commonly used as an ingredient for brewing beer, one of the staples of the Egyptian diet, and bread and beer are often present in funerary assemblages, along with the tools and containers supposedly used for their production [55]. As for other objects, the content identified by the analyses differs from the one described in the inscription on the jar [1], reporting a water offering.

Even the presence of beer would be the most suggestive hypothesis, the emission of sulfur species could be also due to other materials, such as residues of plants from the family of Brassicaceae. Unfortunately, it was not possible to obtain a sample from the content of the amphora S.8224 for microdestructive analysis, thus the interpretation of these data remains inconclusive.

Half of the samples featured a VOCs profile containing specific biomarkers. The profiles were qualitatively compared, and two clusters were identified based on the cluster analysis algorithm.

Differently from the NS cluster, a second group highlighted by the PCA analysis was characterized by the presence of specific VOC markers. This molecular profile was already highlighted in previous studies for other archaeological samples and related to beeswax by comparison of the SIFT-MS and tradition GC/MS results [3], and further characterized by other analytical approaches based on the GC/MS analysis of VOCs emitted from museum objects [19]. The VOC profile of beeswax is mainly characterized by the presence of C_4-C_{11} aliphatic aldehydes, C_3-C_{12} monocarboxylic fatty acids, and long-chain hydrocarbons up to C_{18}. All the mass spectra acquired for the VOCs of the objects

grouped in this cluster were characterized by the presence of these species. As example, **Figure 5** shows the spectra acquired for the headspace of amphora S.8467.

The SIFT-MS mass spectra acquired for the VOCs emitted by amphorae S.8467 and S.8516 showed these features. Moreover, the presence of additional shorter chain monocarboxylic fatty acids (C_3-C_4), either suggested the possible presence of another lipid material, since these VOCs are formed and released during the oxidation undergone by unsaturated fatty acids [56, 57] found in high amounts in plant oils and fats [26, 58, 59], or less likely, that the beeswax underwent a drastic hydrolysis process. A mixture of beeswax and an oil or fat can thus be suggested, either deliberately prepared or derived from the re-use of the jars as a container for the two materials at different times. Interestingly, these two amphoras were positioned in proximity to the entrance of the burial chamber. Similarly to S.8618, they are jars traditionally used for storing and transporting liquids [60] during the New Kingdom between the reign of Amenhotep III and Ramesses III [61]. According to the inscriptions on the three amphorae [1], their primary use was probably to store wine [62]; however, repurposing a container was not uncommon. In ancient Egypt, beeswax was exploited as an ingredient for cosmetics, and ointments [63]. The use of beeswax in mummification balms is attested from the 21st Dynasty, except for an earlier case represented by the beef ribs meat found in Yuya and Thuya tomb dating to the 18th Dynasty [64, 65]. The wide range of applications and symbolic value attributed to beeswax [63] could explain its frequent presence in the tomb of Kha and Merit.

Beeswax markers were also detected in two amorphous lumps (S.8239 and S.8240) probably scooped out of amphora S.8467 by Schiaparelli himself [1]. Consistently, the SIFT mass spectra were very similar to those acquired for jar S.8647, with the presence of short chain fatty acids. To obtain further information and validate the results of the SIFT-MS investigation, we performed the GC/MS on microsamples collected from both the amorphous materials.

Figure 5 reports the SIFT-MS spectra obtained for the headspace of S.8239 and S.8647, together with the GC/MS chromatogram of the extract of the microsample collected from S.8239.

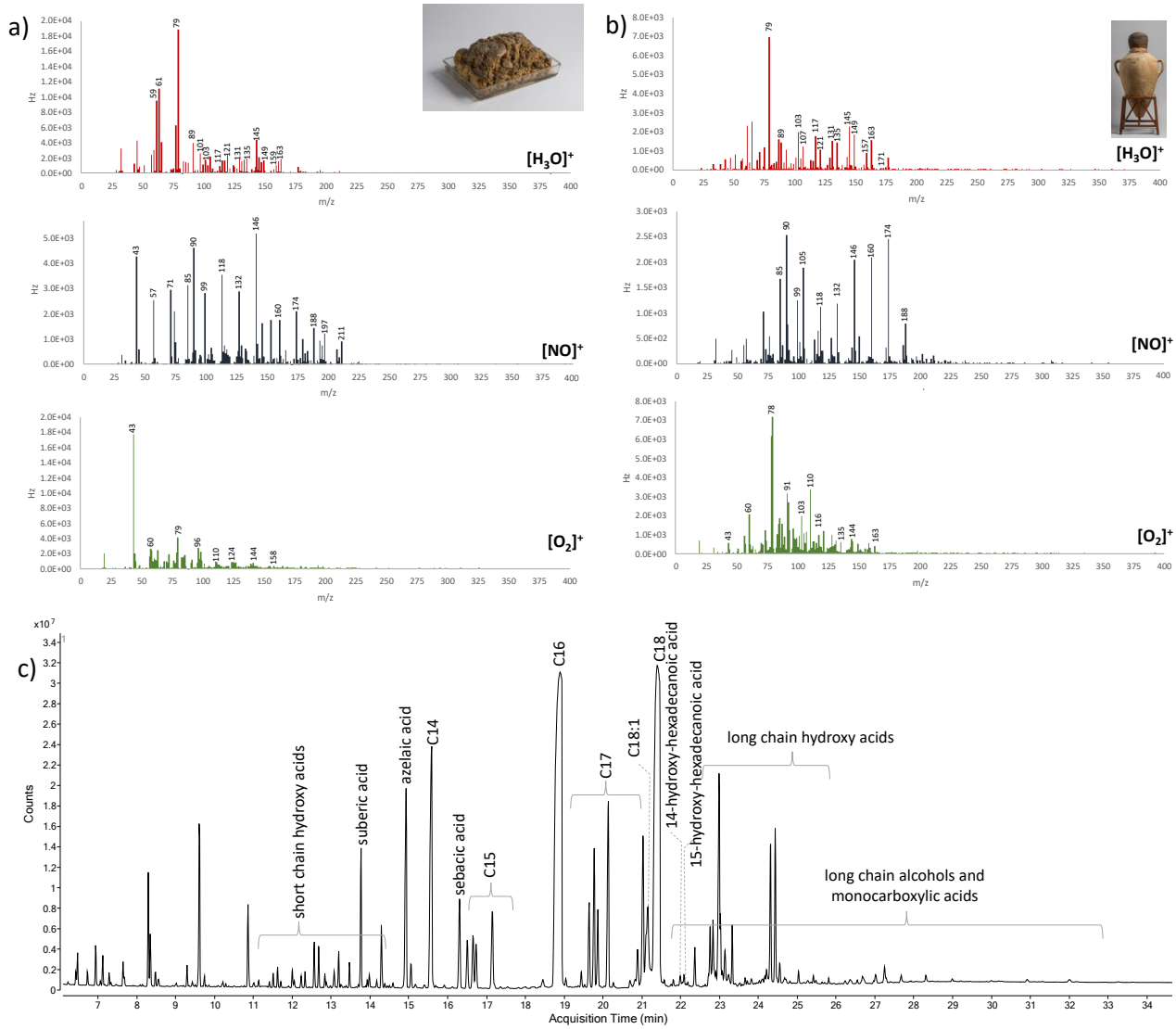


Figure 5 - SIFT-MS mass spectra obtained for the headspace of the amorphous material S.8329 (a) and amphora S.8647 (b), together with the GC-MS chromatogram obtained for the microsample collected from S.8239.

The GC/MS profiles of the two samples were similar (**Figure 5**) and both characterized by the markers of beeswax (palmitic and long chain monocarboxylic acids**,** 14 and 15-hydroxy-hexadecanoic acids, long chain alcohols, and alkanes). The analysis also highlighted the presence of fatty acids with odd chain lengths (C_9, C_{13}, C_{15}, C_{17}, and C_{19}), long chain hydroxy acids, high amounts of saturated fatty acids, with stearic as the most abundant, and small amounts of dicarboxylic acids. This profile is consistent with animal fat [66]. All these results agree with the hypothesis that S.8239 and S.8240 were scooped out of S.8618.

Notably, the GC/MS analysis confirmed the results of SIFT-MS and contributed to explained why these two samples are not centered in the beeswax cluster, but are located in the intersection of two clusters.

A VOC profile indicating the presence of beeswax was also detected for amphora S.8356, although the main components of its headspace were ammonia, dimethylamine, and diethylamine, which are degradation products of proteins [32]. The SIFT-MS analysis also detected furan and furfural derivatives, possibly deriving from saccharide or polysaccharide substances [41-43]. Schiaparelli described the content of this amphora as salted meat [1], and considering that honey, a saccharide substance, was widely used together with beeswax as a preservative [60], the detected furan and furfural derivatives could be ascribed to the degradation of honey [67-69]. The GC/MS and Py-GC-MS analysis performed on a sample taken from the inside of the amphora confirmed the presence of both beeswax and proteins by the identification of the respective markers. The chromatograms are included in the Supporting Information.

The molecular markers of beeswax were also identified in the VOC profiles emitted by two small alabaster bowls (S.8483 and S.8484). In addition, significant amounts of short-chain C_1-C_4 monocarboxylic acids derived from the oxidation of a further lipid were detected in the headspace of S.8484. The materials identified in these bowls were in agreement with the ingredients used to produce hair ointments, as reported by Dioscorides [70], who describes mixtures of ox, pig, or goose fat and also mentions beeswax as a surfactant in cosmetic formulations. In the VOC profile of S.8484 also naphthalene was detected, which is unspecific and could be related to several sources.

The third cluster located on the left of the scatter plot (**Figure 3**) can be associated to lipids materials, probably oils of fats. The mass spectra of the samples included in this cluster were characterized by the presence of C_1-C_8 monocarboxylic fatty acids and aliphatic aldehydes. The profile is consistent with the oxidation products of polyunsaturated fatty acids, such as linolenic and linoleic acids [56, 57], contained in high amounts in plant seeds, such as linen (*Linum usitatissimum*), sesame (*Sesamum indicum*), safflower (*Carthamus tinctorius*), and desert date (*Balanites aegyptiaca*), but also in fish oil [58, 71-74].

During the New Kingdom, amphorae like S.8619 and S.8620 were commonly used as containers for oils and honey [75, 76]. In effect, the headspace of their content indicated the presence of a lipid material. Moreover, the mass spectra of the headspace of S.8619 also featured peaks characteristic of benzene and benzaldehyde, which are unspecific markers of organic materials.

A lipid material was also highlighted in the VOC profiles of the alabaster vessels S.8624, S.8486, and S.8323 since they contained monocarboxylic fatty acids and aliphatic aldehydes in the C_1-C_7 range. The mass spectra acquired for VOCs from S.8323 also featured a few peaks associated with furan and furfural derivatives, possibly emitted by the piece of linen tied around the neck of the alabaster amphora, and peaks ascribable to monoterpenes and toluene, which are specific of a plant aromatic resin such as benzoin or benzoe, probably mixed with the oil/fat [36]. The mass spectra related to VOCs from S.8486 also featured acetone and acetic acid in high amounts.

The investigated objects included a group of seven alabaster vessels (S.8441, S.8442, S.8443, S.8444, S.8445, S.8447, S.8448), possibly related to the “seven sacred oils”. These oils were used during ceremonies, daily temple rituals, and mummification practices [70]. The VOC profiles of this group of vessels showed similar features, suggesting, as with the previous objects, the occurrence of a lipid material containing polyunsaturated fatty acids.

The profiles of S.8441, S.8442, S.8443, S.8445, and S.8447 all featured monocarboxylic fatty acids and aliphatic aldehydes (C_1-C_9) in the same relative amounts, indicating the same oil or fat in all of them. Even if C_5-C_9 monocarboxylic acids are typical of both oils and beeswax (accounting for the partial overlap of the two clusters), the absence of peaks related to aldehydes and carboxylic acids with a chain length from C_{10} up to C_{12}, and of hydrocarbons in these samples excludes the presence of beeswax. The mass spectra obtained for S.8444 and S.8448 contained monocarboxylic fatty acids and aliphatic aldehydes in a different range (C_1-C_7), indicating a different oil or fat from the one stored in the other five vessels. The mass spectra of sample S.8444 contained peaks due to monoterpenes, typical of a plant resin, and several furfural derivatives, and – as discussed for S.8323 – probably deriving from the degradation of the linen cloth around the neck of the jar.

During the SIFT-MS analysis of the alabaster containers S.8443, S.8444, S.8447, and S.8448, small amounts of the organic material contained in the objects and adhered on the cap of the artefacts

sticked on nalophan bags. In **Figure 6** the SIFT-MS mass spectra and GC/MS chromatogram obtained for alabaster S.8444 are reported as example. The gas chromatographic analysis of the organic residues highlighted the presence of fatty acids (up to C_{22}), dicarboxylic acids, and hydroxy fatty acids, compatible with the presence of a lipid material characterized by a relative high degree of oxidation [77]. The presence of a relative high abundance of long and short chain hydroxy fatty acids suggests the presence in the original composition of the material of a high amount of polyunsaturated fatty acids, such as linolenic acid. The analysis of sample S.8444 did not allow to confirm the presence of the resins, probably due to the poor representativeness of the sample which was collected from the outside of the alabaster.

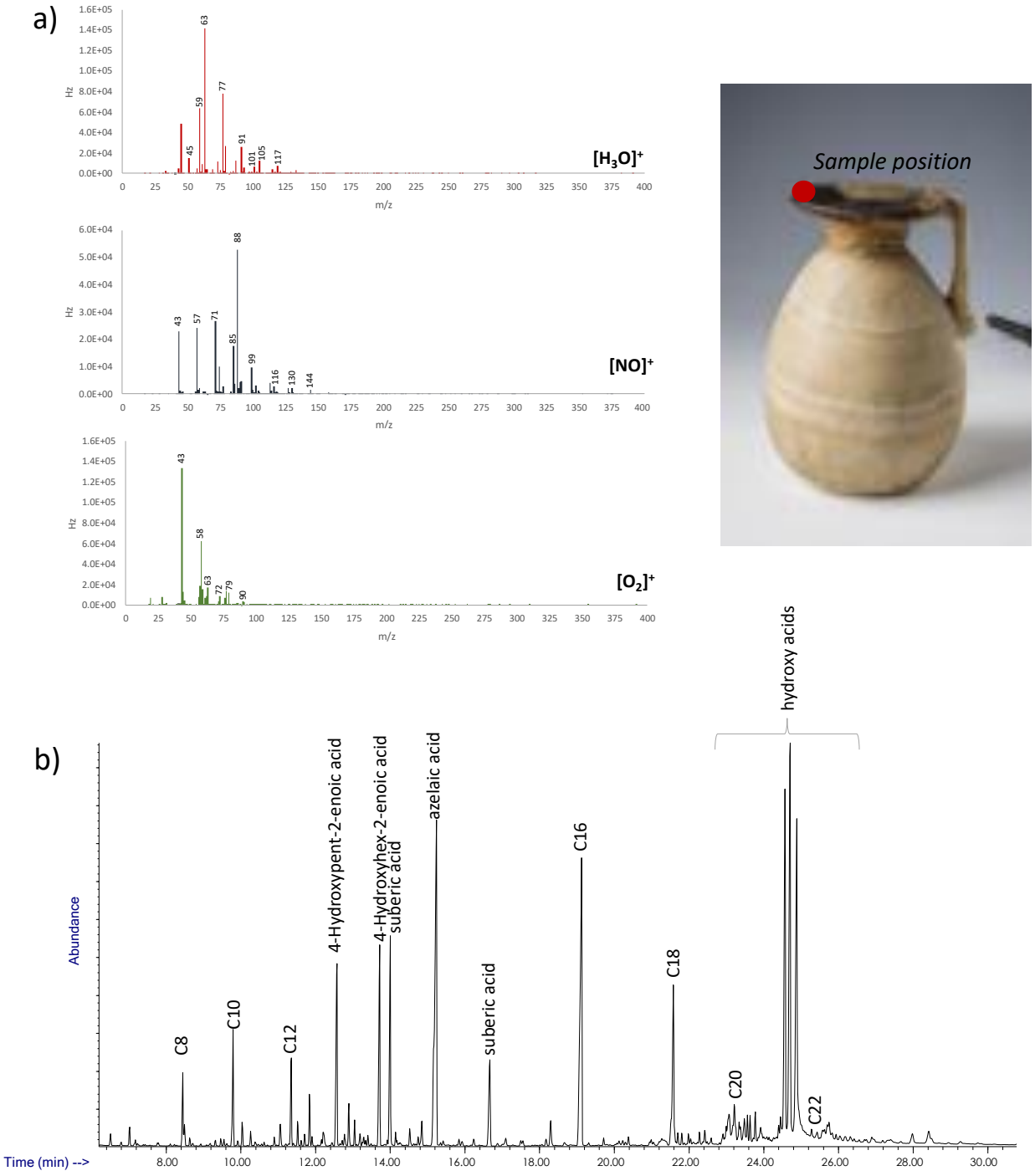


Figure 6 – SIFT-MS mass spectra (a) and GC-MS chromatogram (b) obtained for the sample from S.8444

Conclusions

This work is the first ever extensive non-invasive and non-destructive study based on mass spectrometry on the grave goods of Kha and Merit performed directly in the museum.

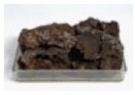
In a wider perspective, the demonstrated in-museum approach expands the potentialities of mass spectrometric studies of archaeological residues in the direction of the non-invasive and non-destructive analysis of large sets of objects.

The use of SIFT-MS sensitive and selective technology, together with the isolation of the containers using inert bags, enabled us to maximize the information obtained from the VOCs emitted from the various objects. The pristine state of conservation of the collection provided the perfect case study for the proof-of-concept of the newly developed SIFT-MS protocol for *in-museum* analysis, moving forward from pilot laboratory scale to in-situ diagnostic campaign. More importantly, we believe that integrating the SIFT-MS results with the archaeological evidence and knowledge significantly advances the understanding of the uses and the chemical composition of the grave goods.

Table 1 summarizes the chemical information of all samples gained from the interpretation of the SIFT-MS mass spectra using chemical ionization with three different reagent ions generated in-situ (H_3O^+, NO^+, O_2^+) together with the Py-GC-MS and GC/MS analysis.

Table 1 - Description and images of the investigated objects, and summary of the molecular markers detected (the most abundant compounds in terms of m/z peak intensity are reported in bold) and material identification hypotheses. The labels applied to each object before cluster analysis are also reported (NS = profile not specific; PROT = protein material; ACET = acetone/acetic acid or acetaldehyde; TOL = toluene as main peak; O/F = Oil or fat material; WAX = beeswax; n.i. = not included in the PCA).

'Museum Inventory Number'	***Object description***	***Pictures***	***Markers***	***Material***	***PCA Label***
S.8219	***Pilgrim flask***		-	Blank	NS

S.8224	***Amphora sealed with linen cloth***		Furan and furfural derivatives, alcohols (C_6-C_8), aldehydes (C_4-C_7), monocarboxylic acids (C_5-C_6), **sulphur compounds**, and linalool	Flour (barley?)	S
S.8233	***Cup with amorphous material***		Non-specific hydrocarbons, furan/furfural derivatives and aldehydes	Unknown	NS
S.8239	***Amorphous lump***		Monocarboxylic acids (C_5-C_{12}), **aldehydes** (C_4-C_{11}), and long chain hydrocarbons up to C_{18}	Beeswax and oil or fat	WAX
S.8240	***Amorphous lump***		Monocarboxylic acids (C_5-C_{12}), **aldehydes** (C_4-C_{11}), and long chain hydrocarbons up to C_{18}	Beeswax and oil or fat	WAX
S.8241	***Amorphous material***		**Aldehydes** (C_2-C_8)	Unknown	NS
S.8246	***Bottle with white granular content***		Acetone, acetaldehyde, and acetic acid	Unknown	ACET
S.8247	***Empty bottle***		Acetone, acetaldehyde, and acetic acid	Unknown	ACET
S.8250	***Miniature amphora sealed with linen cloth***		**Acetaldehyde**	Unknown	ACET
S.8251	***Cup with amorphous material***		**Ammonia**, methylamine, dimethylamine	Protein material	PROT

S.8252	***Cup with dried fruits/seeds***		Non-specific hydrocarbons, furan/furfural derivatives and aldehydes	Unknown	NS
S.8253	***Bowl with dried fruits/seeds***		Non-specific hydrocarbons, furan/furfural derivatives and aldehydes	Unknown	NS
S.8255	***Cup with dried fruits/seeds***		Non-specific hydrocarbons, furan/furfural derivatives and aldehydes	Unknown	NS
S.8256	***Cup with dried fruits/seeds***		Non-specific hydrocarbons, furan/furfural derivatives and aldehydes	Unknown	NS
S.8321	***Cup with amorphous material***		**Ammonia**, methylamine, dimethylamine, trimethylamine and dimethylnitrosoamine	Protein materials (dried fish?)	PROT
S.8323	***Alabaster amphora sealed with linen cloth***		Monocarboxylic acids and aldehydes (both C_1-C_7), furfural derivatives, **toluene** and monoterpenes	Oil or fat containing polyunsaturated fatty acids and aromatic resin	TOL
S.8344	***Cup with dried fruits/seeds***		-	Blank	NS
S.8345	***Cup with dried fruits/seeds***		Non-specific hydrocarbons, furan/furfural derivatives and aldehydes	Unknown	NS
S.8346	***Cup with dried fruits/seeds***		-	Blank	NS
S.8347	***Cup with dried fruits/seeds***		-	Blank	NS

S.8348	***Cup with dried fruits/seeds***		Non-specific hydrocarbons, furan/furfural derivatives and aldehydes	Unknown	NS
S.8349	***Cup with dried fruits/seeds***		Non-specific hydrocarbons, furan/furfural derivatives and aldehydes	Unknown	NS
S.8356	***Amphora containing amorphous material***		**Ammonia**, methylamine, dimethylamine, monocarboxylic acids (C_5-C_{12}), aldehydes (C_4-C_{11}), furan and furfural derivatives	Protein materials and beeswax	PROT
S.8357	***Empty amphora***		**Toluene**	Unknown	TOL
S.8368	***Empty jug***		-	Blank	NS
S.8434	***Jug sealed with linen cloth***		**Acetone**	Unknown	ACET
S.8435	***Jug closed with linen cloth***		-	Blank	NS
S.8441	***Alabaster jar closed with a lid***		**Monocarboxylic acids** and aldehydes (C_1-C_9)	Oil or fat containing polyunsaturated fatty acids	O/F

S.8442	***Alabaster jar sealed with a lid and linen cloth***		**Monocarboxylic acids** and aldehydes (C_1-C_9)	Oil or fat containing polyunsaturated fatty acids	O/F
S.8443	***Alabaster jar sealed with linen cloth***		**Monocarboxylic acids** and aldehydes (C_1-C_9)	Oil or fat containing polyunsaturated fatty acids	O/F
S.8444	***Alabaster jar sealed with linen cloth***		**Monocarboxylic acids** and aldehydes (C_1-C_7), and monoterpenes	Oil or fat containing polyunsaturated fatty acids and natural resin	O/F
S.8445	***Alabaster jar closed with a lid***		**Monocarboxylic acids** and aldehydes (C_1-C_9)	Oil or fat containing polyunsaturated fatty acids	O/F
S.8447	***Alabaster jar sealed with linen cloth***		**Monocarboxylic acids** and aldehydes (C_1-C_9)	Oil or fat containing polyunsaturated fatty acids	O/F
S.8448	***Alabaster jar sealed with linen cloth***		**Monocarboxylic acids** and aldehydes (C_1-C_7)	Oil or fat containing polyunsaturated fatty acids	O/F
S.8467	***Amphora closed with a clay stopper containing amorphous material***		Monocarboxylic acids (C_3-C_{12}), **aldehydes** (C_4-C_{11}), and long chain hydrocarbons up to C_{18}	Beeswax and oil or fat	WAX
S.8483	***Alabaster bowl closed with a lid***		Monocarboxylic acids (C_5-C_{12}), **aldehydes** (C_4-C_{11}), and long chain hydrocarbons up to C_{18}	Beeswax	WAX

S.8484	***Alabaster bowl closed with a lid***		Monocarboxylic acids (C_1-C_{12}), aldehydes (C_1-C_{11}), and long chain hydrocarbons, **naphthalene**	Beeswax and oil or fat	WAX
S.8486	***Alabaster jar closed with a lid***		Acetic acid, **acetone**, monocarboxylic acids and aldehydes (both C_1-C_7)	Oil or fat containing polyunsaturated fatty acids	O/F
S.8516	***Amphora closed with a clay stopper containing amorphous material***		Monocarboxylic acids (C_3-C_{12}), **aldehydes** (C_4-C_{11}), and long chain hydrocarbons up to C_{18}	Beeswax and oil or fat	WAX
S.8523	***Empty jar closed with a cup***		Acetic acid and **acetophenone**	Unknown	n.i.
S.8526	***Amphora containing amorphous material***		Ammonia, methylamine, dimethylamine, diethylamine, trimethylamine and dimethylnitrosoamine, monoterpenes, and **toluene**, benzene, ethylbenzene, and benzoic acid	Protein materials (dried fish?) and aromatic resin	TOL
S.8618	***Amphora sealed with a clay stopper***		-	Blank	NS
S.8619	***Amphora sealed with a cup and linen cloth***		Monocarboxylic acids and aldehydes (both C_1-C_8), **Benzene**, Benzaldehyde	Oil or fat containing polyunsaturated fatty acids	O/F
S.8620	***Amphora sealed with a cup and linen cloth***		**Monocarboxylic acids** and aldehydes (both C_1-C_8)	Oil or fat containing polyunsaturated fatty acids	O/F

S.8624	***Alabaster jug closed with a lid***		**Monocarboxylic acids** and aldehydes (both C_1-C_7)	Oil or fat containing polyunsaturated fatty acids	O/F
S.8627	***Cup with dried fruits/seeds***		Non-specific hydrocarbons, furan/furfural derivatives and aldehydes	Unknown	NS

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Author Contributions

JLN: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing (original draft); ID: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation, writing – original draft, writing – review & editing; FM: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, writing – original draft, writing – review & editing; FF: investigation, writing – original draft, writing – review & editing; VT: investigation, methodology, project administration, writing – original draft, writing – review & editing; AC: data curation, funding acquisition, investigation, methodology, resources, validation, writing – review & editing; CG: resources, supervision, writing – review & editing; EF: conceptualization, methodology, project administration, supervision, writing – original draft, writing – review & editing; MPC: conceptualization, funding acquisition, methodology, resources, supervision, writing – review & editing; ER: conceptualization, formal analysis, investigation, methodology, supervision, writing – original draft, writing – review & editing.

Conflict of Interest

The authors declare that they have no competing interests.

References

1. Schiaparelli, E., *Relazione sui lavori Della Missione Archeologica Italiana in Egitto (anni 1903–1920), volume secondo: La Tomba Intatta dell'Architetto Cha Nella Necropoli di Tebe, Torino 1927 = The intact tomb of the architect Kha in the necropolis of Thebes (reprint with english translation)*. 2008, Torino: AdArte.

2. Smith, S.T., *Intact Tombs of the Seventeenth and Eighteenth Dynasties from Thebes and the New Kingdom Burial System.* MDAIK, 1992. **48**: p. 193-231.

3. La Nasa, J., et al., *SIFT-ing archaeological artifacts: Selected ion flow tube-mass spectrometry as a new tool in archaeometry.* Talanta, 2020. **207**: p. 120323.

4. La Nasa, J., et al., *Validation Study of Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) in Heritage Science: Characterization of Natural and Synthetic Paint Varnishes by Portable Mass Spectrometry.* Journal of The American Society for Mass Spectrometry, 2019.

5. Bonaduce, I., et al., *Analytical Approaches Based on Gas Chromatography Mass Spectrometry (GC/MS) to Study Organic Materials in Artworks and Archaeological Objects.* Topics in Current Chemistry, 2016. **374**(1): p. 6.

6. Ribechini, E., et al., *An integrated analytical approach for characterizing an organic residue from an archaeological glass bottle recovered in Pompeii (Naples, Italy).* Talanta, 2008. **74**(4): p. 555-561.

7. Evershed, R.P., *Organic residue analysis in archaeology: The archaeological biomarker revolution*.* Archaeometry, 2008. **50**(6): p. 895-924.

8. La Nasa, J., F. Modugno, and I. Degano, *Liquid chromatography and mass spectrometry for the analysis of acylglycerols in art and archeology.* Mass Spectrometry Reviews, 2020. **n/a**(n/a).

9. Festa, G., et al., *Egyptian metallic inks on textiles from the 15 th century BCE unravelled by non-invasive techniques and chemometric analysis.* Scientific reports, 2019. **9**(1): p. 1-8.

10. Romano, F.P., Caliri, C., Cavaleri, T., Gargano, M., Buscaglia, P., Ferraris, E., *Investigating the nature and use of manganese black on the grave goods of Kha and Merit (Egypt, 1450-1400 BC) by combining MA-XRF, XRD and multispectral imaging.* in preparation, 2020.

11. Andreani, C., et al., *A neutron study of sealed pottery from the grave-goods of Kha and Merit.* Journal of Analytical Atomic Spectrometry, 2017. **32**(7): p. 1342-1347.

12. Festa, G., et al., *Egyptian grave goods of Kha and Merit studied by neutron and gamma techniques.* Angewandte Chemie, 2018. **130**(25): p. 7497-7501.

13. Fenech, A., et al., *Volatile aldehydes in libraries and archives.* Atmospheric Environment, 2010. **44**(17): p. 2067-2073.

14. Strlič, M., et al., *Material Degradomics: On the Smell of Old Books.* Analytical Chemistry, 2009. **81**(20): p. 8617-8622.

15. Curran, K. and M. Strlič, *Polymers and volatiles: Using VOC analysis for the conservation of plastic and rubber objects.* Studies in Conservation, 2015. **60**(1): p. 1-14.

16. Curran, K., et al., *The development of a SPME-GC/MS method for the analysis of VOC emissions from historic plastic and rubber materials.* Microchemical Journal, 2016. **124**: p. 909-918.

17. Curran, K., et al., *Classifying Degraded Modern Polymeric Museum Artefacts by Their Smell.* Angewandte Chemie International Edition, 2018. **57**(25): p. 7336-7340.

18. Lattuati-Derieux, A., et al., *What do plastics emit? HS-SPME-GC/MS analyses of new standard plastics and plastic objects in museum collections.* Journal of Cultural Heritage, 2013. **14**(3): p. 238-247.

19. Lattuati-Derieux, A., et al., *First results on headspace-solid phase microextraction-gas chromatography/mass spectrometry of volatile organic compounds emitted by wax objects in museums.* Journal of Chromatography A, 2008. **1187**(1-2): p. 239-249.

20. Bleton, J. and A. Tchapla, *SPME/GC-MS in the Characterisation of Terpenic Resins*, in *Organic Mass Spectrometry in Art and Archaeology*. 2009. p. 261-302.

21. Hamm, S., J. Bleton, and A. Tchapla, *Headspace solid phase microextraction for screening for the presence of resins in Egyptian archaeological samples.* Journal of Separation Science, 2004. **27**(3): p. 235-243.

22. Smith, D. and P. Španěl, *Selected ion flow tube mass spectrometry (SIFT‐MS) for on‐line trace gas analysis.* Mass spectrometry reviews, 2005. **24**(5): p. 661-700.

23. Degano, I., et al., *Recent Advances in Analytical Pyrolysis to Investigate Organic Materials in Heritage Science.* Angewandte Chemie International Edition, 2018. **57**(25): p. 7313-7323.

24. La Nasa, J., et al., *Comics' VOC-abulary: Study of the ageing of comic books in archival bags through VOCs profiling.* Polymer Degradation and Stability, 2019. **161**: p. 39-49.

25. Ghimenti, S., et al., *Comparison of sampling bags for the analysis of volatile organic compounds in breath.* Journal of breath research, 2015. **9**(4): p. 047110.

26. Blanco-Zubiaguirre, L., et al., *GC–MS and HPLC-ESI-QToF characterization of organic lipid residues from ceramic vessels used by Basque whalers from 16th to 17th centuries.* Microchemical Journal, 2018. **137**: p. 190-203.

27. Lucejko, J.J., et al., *Long-lasting ergot lipids as new biomarkers for assessing the presence of cereals and cereal products in archaeological vessels.* Scientific Reports, 2018. **8**(1): p. 3935.

28. La Nasa, J., et al., *Synthetic materials in art: a new comprehensive approach for the characterization of multi-material artworks by analytical pyrolysis.* Heritage Science, 2019. **7**(1): p. 8.

29. Ribechini, E., J. Pérez-Arantegui, and M.P. Colombini, *Gas chromatography/mass spectrometry and pyrolysis-gas chromatography/mass spectrometry for the chemical characterisation of modern and archaeological figs (Ficus carica).* Journal of Chromatography A, 2011. **1218**(25): p. 3915-3922.

30. Lee, J., et al., *Scientific investigation into the water sensitivity of twentieth century oil paints.* Microchemical Journal, 2018. **138**: p. 282-295.

31. Carlesi, S., et al., *Multivariate Analysis of Combined Fourier Transform Near-Infrared Spectrometry (FT-NIR) and Raman Datasets for Improved Discrimination of Drying Oils.* Applied Spectroscopy, 2015. **69**(7): p. 865-876.

32. Ge, X., A.S. Wexler, and S.L. Clegg, *Atmospheric amines - Part I. A review.* Atmospheric Environment, 2011. **45**(3): p. 524-546.

33. Sen, N.P., *The evidence for the presence of dimethylnitrosamine in meat products.* Food and Cosmetics Toxicology, 1972. **10**(2): p. 219-223.

34. Fong, Y.Y. and W.C. Chan, *Dimethylnitrosamine in chinese marine salt fish.* Food and Cosmetics Toxicology, 1973. **11**(4): p. 841-845.

35. Gram, L. and P. Dalgaard, *Fish spoilage bacteria – problems and solutions.* Current Opinion in Biotechnology, 2002. **13**(3): p. 262-266.

36. Fernandez, X., et al., *Volatile constituents of benzoin gums: Siam and Sumatra. Part 1.* Flavour and Fragrance Journal, 2003. **18**(4): p. 328-333.

37. Orsini, S., F. Parlanti, and I. Bonaduce, *Analytical pyrolysis of proteins in samples from artistic and archaeological objects.* Journal of Analytical and Applied Pyrolysis, 2017. **124**: p. 643-657.

38. Modugno, F., E. Ribechini, and M.P. Colombini, *Aromatic resin characterisation by gas chromatography–mass spectrometry: Raw and archaeological materials.* Journal of Chromatography A, 2006. **1134**(1): p. 298-304.

39. Ackman, R.G. and R.P. Hansen, *The occurrence of diastereomers of phytanic and pristanic acids and their determination by gas-liquid chromatography.* Lipids, 1967. **2**(5): p. 357-362.

40. Evershed, R.P., et al., *Experimental evidence for the processing of marine animal products and other commodities containing polyunsaturated fatty acids in pottery vessels*.* Archaeometry, 2008. **50**(1): p. 101-113.

41. Lattuati-Derieux, A., S. Bonnassies-Termes, and B. Lavédrine, *Identification of volatile organic compounds emitted by a naturally aged book using solid-phase microextraction/gas chromatography/mass spectrometry.* Journal of Chromatography A, 2004. **1026**(1): p. 9-18.

42. de Pablo, A., *Furfural and ageing: how are they related.* 1999.

43. Ott, E., et al., *Cellulose and cellulose derivatives*. Vol. 5. 1954: Interscience Publishers.

44. Defilippi, B.G., et al., *Chapter 1 Aroma Volatiles: Biosynthesis and Mechanisms of Modulation During Fruit Ripening*, in *Advances in Botanical Research*. 2009, Academic Press. p. 1-37.

45. Hatanaka, A., *The fresh green odor emitted by plants.* Food Reviews International, 1996. **12**(3): p. 303-350.

46. O'Neil, M.J., *The Merck index: an encyclopedia of chemicals, drugs, and biologicals*. 2013: RSC Publishing.

47. Arnette, M.-L., *Les dépôts alimentaires dans la tombe de Khâ (TT 8): synthèse et tentative d'approche symbolique*, in *Life, Death, and Coming of Age in Antiquity : Individual Rites of Passage in the Ancient Near East and Adjacent Regions*, J.P. Alice Mouton, Editor. 2014, PIHANS Leiden p. 336-390.

48. Klensporf, D. and H.H. Jeleń, *Analysis of volatile aldehydes in oat flakes by spme-gc/ms.* Polish Journal of Food and Nutrition Sciences, 2005. **55**(4): p. 389-395.

49. Prosen, H., et al., *Comparison of isolation methods for the determination of buckwheat volatile compounds.* Food Chemistry, 2010. **121**(1): p. 298-306.

50. Dong, L., et al., *Analysis of volatile compounds from a malting process using headspace solid-phase micro-extraction and GC–MS.* Food Research International, 2013. **51**(2): p. 783-789.

51. Yang, B., P. Schwarz, and R. Horsley, *Factors Involved in the Formation of Two Precursors of Dimethylsulfide During Malting.* Journal of the American Society of Brewing Chemists, 1998. **56**(3): p. 85-92.

52. Hysert, D.W., N.M. Morrison, and R.L. Weaver, *Dimethyl Sulfide Precursor in Beer.* Journal of the American Society of Brewing Chemists, 1979. **37**(4): p. 169-174.

53. Burmeister, M.S., et al., *Measurement of Volatile Sulfur Compounds in Beer Using Gas Chromatography with a Sulfur Chemiluminescence Detector.* Journal of the American Society of Brewing Chemists, 1992. **50**(2): p. 53-58.

54. A. Mouton and A. Mouton, *Life, Death, and Coming of Age in Antiquity : Individual Rites of Passage in the Ancient Near East and Adjacent Regions*, ed. A. Mouton and A. Mouton. 2014, Lovanio: Peeters Publishers.

55. Farag, M.A., et al., *Revealing the constituents of Egypt's oldest beer using infrared and mass spectrometry.* Scientific Reports, 2019. **9**(1): p. 16199.

56. Juita, et al., *Identification and Quantitation of Volatile Organic Compounds from Oxidation of Linseed Oil.* Industrial & Engineering Chemistry Research, 2012. **51**(16): p. 5645-5652.

57. Fjällström, P., et al., *Drying of linseed oil paints: a laboratory study of aldehyde emissions.* Industrial Crops Products, 2002. **16**(3): p. 173-184.

58. La Nasa, J., et al., *Core shell stationary phases for a novel separation of triglycerides in plant oils by high performance liquid chromatography with electrospray-quadrupole-time of flight mass spectrometer.* Journal of Chromatography A, 2013. **1308**: p. 114-124.

59. Bell, M., R. Henderson, and J. Sargent, *The role of polyunsaturated fatty acids in fish.* Comparative Bioche mistry Physiology Part B: Comparative Biochemistry, 1986. **83**(4): p. 711-719.

60. Ikram, S., *Choice cuts: meat production in ancient Egypt*. Vol. 69. 1995: Peeters Publishers.

61. Hope, C., *Three Studies. Victoria College Archaeological Research Unit Occasional Paper. Pottery of the Egyptian New Kingdom.* Occasional Paper 2, 1989(2).

62. Mcgovern, P.E., *Wine of Egypt's Golden Age: An Archaeochemical Perspective.* 1997. **83**(1): p. 69-108.

63. Raven, M.J., *Wax in Egyptian magic and symbolism*. Oudheidkundige Mededelingen Leiden. Vol. 64. 1983. 7-47.

64. Clark, K.A., *Tracing the evolution of organic balm use in Egyptian mummification via molecular and isotopic signatures.* Phd Thesis, University of Bristol, 2006.

65. Henderson, J., et al., *Ancient Egyptian materials and technology*. 2000: Cambridge University Press.

66. Evershed, R.P., et al., *Chemistry of Archaeological Animal Fats.* Accounts of Chemical Research, 2002. **35**(8): p. 660-668.

67. Giordano, L., et al., *Quantitative analysis of 2-furfural and 5-methylfurfural in different Italian vinegars by headspace solid-phase microextraction coupled to gas chromatography–mass spectrometry using isotope dilution.* Journal of Chromatography A, 2003. **1017**(1): p. 141-149.

68. Wolski, T., et al., *Identification of honey volatile components by solid phase microextraction (SPME) and gas chromatography/mass spectrometry (GC/MS).* Journal of Apicultural Science, 2006. **50**(2): p. 115-126.

69. Ozcan-Sinir, G., O.U. Copur, and S.A. Barringer, *Botanical and geographical origin of Turkish honeys by selected-ion flow-tube mass spectrometry and chemometrics.* 2020. **100**(5): p. 2198-2207.

70. Manniche, L., *Sacred luxuries: fragrance, aromatherapy, and cosmetics in ancient Egypt*. 1999: Cornell University Press.

71. Uzun, B., Ç. Arslan, and Ş. Furat, *Variation in Fatty Acid Compositions, Oil Content and Oil Yield in a Germplasm Collection of Sesame (Sesamum indicum L.).* Journal of the American Oil Chemists' Society, 2008. **85**(12): p. 1135-1142.

72. Rzóska, J., *The Nile, biology of an ancient river: biology of an ancient river*. Vol. 29. 2012: Springer Science & Business Media.

73. Leone, A., et al., *Moringa oleifera Seeds and Oil: Characteristics and Uses for Human Health.* International journal of molecular sciences, 2016. **17**(12): p. 2141.

74. Mohamed, A.M., W. Wolf, and W.E.L. Spieß, *Physical, morphological and chemical characteristics, oil recovery and fatty acid composition of Balanites aegyptiaca Del. kernels.* Plant Foods for Human Nutrition, 2002. **57**(2): p. 179-189.

75. Van Dijk, J., *Hieratic inscriptions from the tomb of Maya at Saqqara: a preliminary survey.* GöttMisz, 1992. **127**: p. 23-32.

76. Aston, D., *A taste of honey : mnt- and mḏqt-vessels in the late Eighteenth Dynasty*, in *Egyptian Stories A British Egyptological Tribute to Alan B. Lloyd on the Occasion of his Retirement*, K.M.S. Thomas Schneider, Alan B Lloyd, Editor. 2007, Ugarit-Verlag: Münster.

77. Andreotti, A., et al., *Combined GC/MS Analytical Procedure for the Characterization of Glycerolipid, Waxy, Resinous, and Proteinaceous Materials in a Unique Paint Microsample.* Analytical Chemistry, 2006. **78**(13): p. 4490-4500.